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学 位 論 文

Significance of myocardial tenascin-C expression in
left ventricular remodeling and long-term outcome
in patients with dilated cardiomyopathy

(拡張型心筋症における心筋内テネイシン C 発現の
左室リモデリングと長期予後に対する意義)

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**Significance of myocardial tenascin-C expression in left ventricular remodeling and
long-term outcome in patients with dilated cardiomyopathy**

Brief Title: Myocardial tenascin-C in dilated cardiomyopathy

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和文要旨

背景：拡張型心筋症は、心筋細胞の変性や間質線維化により、心室の拡大および収縮不全を来す疾患である。遺伝的素因、ウイルス感染、自己免疫異常の関与が指摘されているが、病因は依然不明である。また、拡張型心筋症は予後不良の疾患であり、1年間の死亡率が10%~30%と報告され、心移植の適応となる症例もいる。テネイシンCは細胞外マトリックス蛋白の1種であり、組織障害や腫瘍の形成などの組織のリモデリングに伴い出現する。心疾患においては、心筋梗塞や心筋炎などの病的状態で心臓の組織に出現することが知られている。しかし、拡張型心筋症における心筋内テネイシンC発現の意義については明らかになってはいない。そのため、本研究では拡張型心筋症における心筋内テネイシンC発現について検討した。方法：国立循環器病研究センターにおいて、2005年から2008年までに右室心筋生検を施行され拡張型心筋症と診断された123例を対象とし、後ろ向きに検討した。保存されている心筋生検標本に対してテネイシンC(4F10)による免疫染色を行い、解析ソフトを用いて半定量的に心筋組織内のテネイシンC発現量を解析し、臨床像や予後との関連を検討した。結果：総死亡を対象としてROC解析を行い、心筋内テネイシンC発現量に基づいて対象を2群に分けた（発現量が2.3%以上：テネイシンC高値群、 $n = 22$ 。発現量が2.3%未満：テネイシンC低値群、 $n = 101$ ）。テネイシンC高値群ではテネイシンC低値群と比較して、心不全の罹患歴が長く、糖尿病が多く、肺動脈楔入圧や平均肺動脈圧がより高値で、心筋線維化の割合が多かった。66±35か月の観察期間において、テネイシンC高値群はテネイシンC低値群に比較して有意に死亡例が多かった（ $P < 0.001$ ）。また、テネイシンCは多変量解析において、独立して総死亡と関連していた（ハザード比 1.347、 $P = 0.032$ ）。さらに左室リモデリングについて、診断後約9か月後の心エコー検査における左室内径、左室駆出率を調査し検討したところ、左室内径と左室駆出率の改善率はテネイシンC高値群で有意に少なかった。考察：拡張型心筋症は予後不良の疾患であり、予後予測因子を解明することは重要である。また、拡張型心筋症の左室リモデリングの詳細な機序については不明な点も多い。テネイシンCは組織のリモデリングと関連していると報告されている細胞外マトリックス蛋白であるが、これまで心筋内テネイシンC発現の拡張型心筋症における意義は不明であった。本研究により、拡張型心筋症において心筋内テネイシンC発現高値は、左室リモデリングの改善が不良であることや、予後不良と独立して関連していることが初めて明らかとなった。心筋生検により心筋内テネイシンC発現を調べることで、拡張型心筋症の左室リモデリングや予後を予測できる可能性が示唆された。

Abstract

Aim: Dilated cardiomyopathy (DCM) has a variety of causes, and no useful approach to predict left ventricular (LV) remodeling and long-term outcome has yet been established. Myocardial tenascin-C (TNC) is known to appear under pathological conditions, possibly to regulate cardiac remodeling. The aim of this study was to clarify the significance of myocardial TNC expression in LV remodeling and the long-term outcome in DCM.

Methods and Results: One hundred and twenty-three consecutive DCM patients who underwent endomyocardial biopsy for initial diagnosis were studied. Expression of TNC in biopsy sections was immunohistochemically analyzed to quantify the ratio of the TNC-positive area to the whole myocardial tissue area (TNC area). Clinical parameters associated with TNC area were investigated. The patients were divided into two groups based on receiver operating characteristic analysis of TNC area to predict death; high TNC group with TNC area $\geq 2.3\%$ (22 patients) and low TNC group with TNC area $< 2.3\%$ (101 patients). High TNC was associated with diabetes mellitus. Comparing echocardiographic findings between before and 9 months after endomyocardial biopsy, the low TNC group was associated with decreased LV end-diastolic diameter and increased LV ejection fraction, whereas the high TNC group was not. Survival analysis revealed a worse outcome in the high TNC group than in the low TNC group ($P < 0.001$). Multivariate Cox regression analysis revealed that TNC area was independently associated with poor outcome (HR = 1.347, $P = 0.032$).

Conclusions: Myocardial TNC expression was associated with worse LV remodeling and long-term outcome in DCM.

Key Words: dilated cardiomyopathy ▪ heart failure ▪ tenascin-C ▪ left ventricular remodeling ▪ prognosis

Introduction

Dilated cardiomyopathy (DCM) is characterized by ventricular enlargement and impaired contractile function. The prognosis of DCM remains unfavorable, with a high incidence of sudden cardiac death and heart failure and an estimated overall 1-year mortality rate between 10 and 30 percent, and some patients with DCM require heart transplantation.^{1, 2} Because of the widely diverse background characteristics of patients with DCM, the outcome of these patients is almost unpredictable, even with a variety of examinations including blood tests, electrocardiography, chest X-ray, echocardiography, scintigraphy, and cardiac catheterization. Therefore, new prognostic indicators to stratify the future risk of DCM are strongly awaited.

Tenascin-C (TNC), a large hexameric glycoprotein, is known to appear in the extracellular matrix specifically following tissue injury or tumor formation where the particular organ is undergoing remodeling.³ As regards the cardiac system, TNC is synthesized and expressed under pathological conditions in myocardial infarction, acute myocarditis, and DCM, suggesting a potential impact on progression of cardiovascular diseases.^{4, 5} For example, TNC exacerbated heart failure by acceleration of left ventricular (LV) remodeling and an increase in fibrosis after myocardial infarction in a mouse model.⁶ However, the effect of myocardial TNC expression on LV remodeling and long-term outcome in DCM has not yet been fully investigated. Similarly, the clinical background in

which expression of TNC is increased in the myocardium has not been elucidated, although TNC is known to be synthesized by interstitial fibroblasts under inflammatory stimulation.³

The objective of this study was to investigate the association of myocardial TNC expression with LV remodeling and the long-term outcome. In addition, we examined the clinical factors of patients predisposed to increased tissue TNC expression in DCM.

Methods

Study population

Patients who were diagnosed with DCM in our hospital between 2005 and 2008 with impaired contractile function with left ventricular ejection fraction (LVEF) <40% and left ventricular end-diastolic diameter (LVEDD) >55 mm were screened for the study. All patients underwent clinical evaluation, physical examination, 12-lead electrocardiography, laboratory tests, echocardiography, and cardiac catheterization including coronary angiography and right ventricular (RV) septal endomyocardial biopsy for initial diagnosis. All patients underwent right ventricular endomyocardial biopsy according to the indications in the American Heart Association, the American College of Cardiology, and the European Society of Cardiology scientific statement, mainly to exclude myocarditis or secondary cardiomyopathy.⁷ Endomyocardial biopsy was performed after obtained written informed consent about the procedure and risk of the endomyocardial biopsy. Because medical therapy was optimized during hospitalization in most of the patients, information about use of medication for heart failure such as β -blockers, angiotensin converting enzyme (ACE)-inhibitors or angiotensin receptor blockers (ARB), and loop diuretics was collected separately on admission and at discharge. Patients with hypertrophic cardiomyopathy, ischemic cardiomyopathy, valvular heart disease, relevant LV hypertrophy suggested to be caused by hypertensive heart disease, or secondary cardiomyopathy such as that caused by sarcoidosis or amyloidosis were excluded.

Patients with a history of uncontrolled hypertension or LV assist device implantation were also excluded.

Study protocol

Information about the patients' baseline characteristics, such as age, gender, body mass index, presenting symptoms, blood pressure, heart rate, previous comorbid conditions, and laboratory data (e.g. BNP, hemoglobin, serum creatinine), were collected from their medical records. Data on medication were collected both on admission and at discharge. Sections from stored paraffin-embedded RV septal tissue were stained with a specific antibody to TNC to evaluate TNC expression in the myocardium. Echocardiography was performed before and at 9 months after RV biopsy. Survival data were also collected in order to perform survival analysis. This study was performed according to the Helsinki Declaration and was approved by the ethics committee of our institution.

Heart failure risk score

To evaluate heart failure severity, we used heart failure risk score accessible by the website www.heartfailurerisk.org.⁸ This is the generalizable easily used integer risk score for mortality in patients with heart failure, including 13 independent predictors: age, LVEF, New York Heart Association (NYHA) class, serum creatinine, diabetes mellitus, β -blocker,

systolic blood pressure, body mass index, time since diagnosis, current smoker, chronic obstructive pulmonary disease, male gender, and ACE inhibitor or ARB.

Echocardiography

Echocardiographic parameters were measured according to the recommendations of the American Society of Echocardiography.⁹ LVEDD, left ventricular end-systolic diameter (LVESD), thickness of the interventricular septum and posterior ventricular wall, and left atrial diameter were obtained from M-mode or 2-dimensional images of parasternal long axis views. Left ventricular end-diastolic diameter index (LVEDDI) was LVEDD divided by body surface area. LVEF was calculated by the Teichholz formula.¹⁰ Mitral regurgitation was semiquantitatively graded from 1 to 4 considering the regurgitant area at color doppler imaging. Peak early (E), late (A) diastolic transmitral filling velocities, and deceleration time of E were measured, and E/A ratio was calculated. Restrictive filling pattern was defined as the E-wave deceleration time of <115 ms.¹¹

Cardiac catheterization

Patients underwent diagnostic cardiac catheterization. Coronary angiography was performed to exclude ischemic heart disease, and right heart catheterization was performed to obtain hemodynamic data including cardiac output, pulmonary capillary wedge

pressure (PCWP), and pulmonary artery pressure (PA). Endomyocardial biopsy was also performed, and 3 to 5 pieces of ventricular tissue were obtained from different locations in the septal wall of the right ventricle using disposable biopsy forceps (Technowood Co., Ltd., Tokyo, Japan). The specimens were immediately fixed with 10% buffered formalin, embedded in paraffin, serially sectioned and stained with hematoxylin and eosin staining. Masson's trichrome staining was also performed to detect fibrosis.

Immunohistochemistry for TNC

Immunohistochemical staining on formalin-fixed paraffin-embedded sections was performed using an autostainer Leica Bond-III (Leica Biosystems, Melbourne, Australia) according to the manufacturer's protocol. For antigen retrieval, slides were treated with Enzyme 1 using a Bond Enzyme Pretreatment Kit (AR9551, Leica Biosystems, Newcastle, UK) for 5 min at 37°C. Slides were then incubated for 15 min at room temperature with a commercially available mouse monoclonal antibody for TNC (4F10TT; Immuno-Biological Laboratory Co., Ltd., Gunma, Japan; 1:1000 dilution), which recognizes EGF-like domain, constitutive sites of the TNC molecules.¹² Antibody detection and counterstaining with hematoxylin were performed using Bond Polymer Refine Detection Kit (DS9800, Leica Biosystems, Newcastle, UK). Non-immunized mouse IgG (X0931, 1:1000 dilution; Dako, Glostrup, Denmark) was substituted as a negative control for the primary antibody against

TNC to exclude possible false-positive responses from the secondary antibody or from non-specific binding of IgG.

Analysis of myocardial collagen accumulation and tenascin-C expression

Myocardial collagen accumulation and TNC expression were semi-quantitatively measured in a blinded manner. Photomicrographs were taken in five different randomly-selected high-power fields in the low magnification whole view of three to five pieces obtained by endomyocardial biopsy. Images were analyzed with National Institutes of Health imaging software. The ratio of total blue stained area without endocardium and blood vessels to the whole myocardial area in Masson's trichrome-stained sections was calculated. The average value was taken to represent collagen accumulation and defined as the collagen area. Similarly, the TNC area was expressed as the average of the ratio of TNC-positive area to the whole myocardial area in immunohistochemically stained sections.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences version 21.0 (SPSS Inc.; Chicago, IL, USA). All quantitative data were expressed as mean \pm SD. The statistical significance of differences was analyzed using unpaired Student's t test for parametric continuous variables, Mann-Whitney U test for nonparametric continuous variables, and

paired t-test to compare echocardiographic parameters at baseline and follow-up.

Categorical variables were compared using Fisher's exact test. Pearson's or Spearman's correlation analysis was performed to estimate correlations between TNC area and fibrosis area. The receiver operating characteristic curve was constructed to determine the cut-off values. For survival analysis, the outcome of interest was death, and other patients were censored at the time of LV assist device implantation or heart transplantation, or at the day of last follow-up. Event-free survival curves were constructed using the Kaplan-Meier method, and the statistical significance of differences between curves was assessed using the log-rank test. Cox proportional hazard analysis was performed with 22 clinical variables (age, body mass index, current smoke, NYHA class III or IV, duration of heart failure, heart rate, systolic blood pressure, diabetes mellitus, implantable cardioverter defibrillator [ICD] or biventricular ICD, logBNP, hemoglobin, estimated glomerular filtration rate, β -blocker at discharge, ACE inhibitor or ARB at discharge, aldosterone inhibitor at discharge, loop diuretics at discharge, LVEDDI, LVEF, mitral regurgitation, restrictive filling pattern, heart failure risk score, fibrosis area) and TNC area, and variables achieving $p < 0.05$ on univariable analysis were then tested in multivariable Cox analysis to determine which ones were significantly associated with death.⁸ Univariable linear regression analysis for TNC area was performed using all variables in the baseline characteristics, and then multivariable linear regression analysis was performed using the

variables achieving $P < 0.05$ on univariable linear regression analysis to determine the factors associated with TNC area. Values of $P < 0.05$ were considered statistically significant.

Results

Patient clinical characteristics

A total of 138 consecutive patients were finally diagnosed with DCM, and of them, 123 patients with complete data collection were included in the study. On admission, 43% and 63% of patients were receiving β -blockers and ACE inhibitors/ARBs respectively, but, at discharge, most of the patients were receiving these key medications for heart failure, suggesting that medications were optimized during hospitalization (Table 1). During the follow-up period of 66 ± 35 months, nine patients (7.3%) died, eight patients (6.5%) underwent LV assist device implantation. No patient underwent heart transplantation before LV assist device implantation.

Myocardial collagen area and myocardial tenascin-C expression

A representative image of Masson's trichrome-stained section is shown in Figure 1A (collagen area 30.5%). Collagen area ranged from 0.35 to 44.9% (mean $14.4 \pm 7.7\%$).

Representative images of TNC-stained histological sections with high (Figure 1B, TNC area 9.9%) and low TNC (Figure 1C, TNC area 0.5%) expression are also shown. The distribution of TNC area is depicted in Figure 2. TNC area ranged from 0.1 - 10.4% (mean $1.4 \pm 2.0\%$), with more than 80% of all patients having TNC area $\leq 2\%$. The TNC area was positively correlated with collagen area ($r = 0.329$, $p < 0.001$, Figure 3). Based on receiver operating characteristic analysis, the optimal cut-off value of TNC area to predict all-cause

death in patients with DCM was determined to be 2.3% (sensitivity = 0.600, specificity = 0.858, area under the curve = 0.773, $p = 0.004$; Figure 4) in these study subjects. We divided the patients into two groups using this cut-off value; (i) high TNC group with TNC area $\geq 2.3\%$ ($n = 22$, 18%), and (ii) low TNC group with TNC area $< 2.3\%$ ($n = 101$, 82%).

Patient characteristics and tenascin-C expression

Baseline characteristics were compared between the two groups (Table 1). Higher TNC expression was associated with longer duration of heart failure, higher incidence of diabetes mellitus, a loop diuretic at discharge, and an ACE inhibitor or ARB at discharge. Mean PCWP, mean PA, plasma BNP level on admission, and collagen area in the high TNC group were higher than those in the low TNC group. However, baseline NYHA class, LV dimensions, LVEF, and heart failure risk score were not significantly different between the groups.

Association of tenascin-C expression with outcome

Kaplan-Meier survival curves demonstrated that the high TNC group had a poorer outcome than the low TNC group ($P < 0.001$; Figure 5). Univariable and multivariable Cox regression analyses were performed to determine predictive factors for death. Systolic blood pressure, diabetes mellitus, ICD or biventricular ICD, log BNP, collagen area, and

TNC area with a P value < 0.05 were selected by univariable analysis. Among these, multivariable analysis revealed that TNC area (hazard ratio = 1.347, P = 0.032) was an independent predictor of death (Table 2).

Left ventricular remodeling

One patient died and two others had an LV assist device implanted before follow-up echocardiography. Follow-up echocardiographic data were unavailable in 27 patients. A total of 94 patients (15 patients in the high TNC group and 79 patients in the low TNC group) underwent follow-up echocardiography, performed 9 ± 4 months after the diagnosis of DCM. We found that LVEDD, LVESD and LAD were significantly decreased ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively), and LVEF and deceleration time were significantly increased ($P < 0.001$, $P < 0.001$, respectively) at follow-up compared with baseline in the low TNC group, while these values did not differ between baseline and follow-up in the high TNC group (Table 3). To compare LV remodeling between the high and low TNC groups, we evaluated the changes in echocardiographic parameters from baseline to follow-up. Changes in LVEDD ($P = 0.041$) and LVESD ($P = 0.010$) were significantly smaller in the low TNC group, and change in LVEF ($P = 0.003$) was greater in the low TNC group compared with the high TNC group, suggesting that LV reverse remodeling was less prone to be induced during the follow-up period in the high TNC group (Figure 6).

Determinants of tenascin-C area

Univariable and multivariable linear regression analysis were performed to determine the background characteristics associated with the high TNC area. All variables in the baseline patient characteristics were selected for univariable analysis. Among these variables, diabetes mellitus, ICD or biventricular ICD, mean PCWP, and mean PA were shown to be independent determinants of high TNC area by multivariable analysis (Table 4).

Discussion

In the present study, we found that myocardial TNC expression, immunohistochemically stained and analysed semiquantitatively in RV biopsy samples, was a useful predictor of LV remodeling and long-term outcome.

The outcome of DCM varies greatly among individual cases, and is closely associated with the degree of LV remodeling and whether cardiac function is improved by conventional therapy including β -blockers and ACE-inhibitors.^{13, 14} LV remodeling is affected by various kinds of neurohumoral and local factors such as the renin-angiotensin-aldosterone system, the adrenergic nervous system, increased oxidative stress, proinflammatory cytokines, and endothelin.¹³⁻¹⁷ However, the precise mechanisms that cause LV remodeling are still unclear.

Ventricular extracellular matrix proteins, which maintain cardiac structure and architecture in combination with myocytes, play a central role in LV remodeling in DCM.¹⁸ Recently, several studies have shown that TNC, one of the extracellular matrix component proteins, is expressed in association with several cardiovascular diseases.^{19, 20} This large glycoprotein (> 300 kDa) is expressed only during the development of the embryonic heart and not in the normal adult heart.²¹ However, when exposed to mechanical or ischemic stress, it reappears in the heart. For example, TNC is expressed after myocardial infarction, in acute or chronic myocarditis, and in DCM in response to tissue injury and inflammation.⁴

⁵ Previously, serum TNC level was reported to be related to disease progression and to

have prognostic significance in DCM. Serum measurement of TNC level, because of its less invasive nature, could serve as a potential clinical biomarker reflecting TNC expression.^{19, 20,}

²² In the present study, by directly staining myocardial biopsy specimens, we demonstrated that high TNC expression was related to a worse outcome and lower occurrence of reverse remodeling in patients with DCM. As TNC is not exclusively synthesized in the heart, but also in organs such as the liver and lungs,³ direct histological investigation of myocardial expression using a specific antibody seems to have great significance.

TNC has several functions that weaken cell adhesion, up-regulate the expression and activity of matrix metalloproteinases, modulate inflammatory response, promote recruitment of myofibroblasts, and enhance fibrosis.³ In an experimental myocardial infarction model using genetically altered mice, TNC deficiency ameliorated collagen accumulation in the infarct border area, resulting in improved post-myocardial infarction cardiac function.⁶ TNC has the structural capability to bind and interact with other cells. This large extracellular matrix glycoprotein, consisting of epidermal growth factor (EGF)-like repeats, fibronectin III repeats and a fibrinogen-like domain, has several biological effects. The antibody we used in the current study recognizes the EGF-like domain, and is suitable to measure total myocardial TNC level, because the subsequent fibronectin III domain contains a splice variant region that varies considerably in its

expression form. The EGF-like repeat domain interacts with the epidermal growth factor receptor, and the fibronectin III-like repeat domain binds integrins to promote adhesion and mediates cell activation via toll-like receptor 4, leading to sustained inflammation.²³ The unfavorable effects of TNC on ventricular remodeling in DCM might result from ongoing myocardial damage or inability to repair the failing heart due to sustained inflammation. There is increasing evidence that splicing variants of TNC, especially those containing the fibronectin III B or C domain, may influence tissue remodeling in heart failure. Serum level, as well as cardiac tissue deposition, of the B⁺ TNC variant was associated with mortality in DCM.²² In patients with heart failure, serum levels of B⁺ and C⁺ TNC were associated with heart chamber dilatation, physical performance, and BNP level, suggesting that they are possible biomarkers for disease severity.²⁴ Further immunohistochemical analysis of TNC splicing variants might provide additional valuable information related to LV remodeling.

Another intriguing finding of this study was the relevance of diabetes mellitus to myocardial TNC. Multivariable analysis showed that the presence of diabetes, as well as indices representing heart failure severity such as previous implantation of ICD or biventricular ICD, mean PCWP, and mean PA, was independently associated with myocardial TNC expression. Diabetic cardiomyopathy is a disorder of heart muscle resulting from diabetes, the pathogenesis of which is yet to be clearly defined. Remodeling

of cardiac extracellular matrix is known to be a key pathological feature of diabetic cardiomyopathy. Production of matricellular proteins is increased by hyperglycemia- and hyperinsulinemia-induced chronic inflammation, purportedly through transforming growth factor- β signalling.²⁵ In addition, persistent hyperglycemia induces the formation of advanced glycation end-products, which is associated with increased production and reduced turnover of matricellular proteins.²⁶ Taking into consideration that TNC is likely to be upregulated in an inflammatory microenvironment, the increased myocardial expression of TNC in the present study might have been caused by stimulation of increased transforming growth factor- β associated with diabetes mellitus. We believe that this study also supports the pathophysiological significance of TNC in diabetic cardiomyopathy, in that TNC may contribute to the development of worsening heart function in diabetic patients. Furthermore, from a clinical aspect, it is suggested that preventing or adequately controlling diabetes mellitus might be able to reduce myocardial chronic inflammation, diminish myocardial TNC expression and consequently promote cardiac reverse remodeling in DCM patients.

In the present study, RV endomyocardial biopsy was selected to collect myocardial tissue samples, in order to minimize the procedural risk. It is controversial as to whether RV biopsy specimens are adequate for evaluation of LV remodeling in cardiomyopathy. We consider myocardial TNC measurement using RV myocardial samples is reasonable for the

present study for two reasons. First, samples were obtained from the interventricular septum, which is considered common on the right ventricle and left ventricle. Second, progression of cardiac remodeling in DCM typically results in both RV and LV dysfunction at the same time. In fact, there is evidence that right ventricular dysfunction is correlated with LV dysfunction in patients with DCM.²⁷ We experienced difficulty in RV volumetric and functional assessment because of geometric complexity and asymmetry of the RV.²⁸ Cardiac magnetic resonance imaging might be an option to evaluate RV remodeling.

There are several limitations to this study. First, this was a retrospective study that included a relatively small number of patients. Follow-up echocardiography was not performed in all patients, although 76% of the study population did undergo this procedure. Larger prospective studies with complete follow-up are needed to establish evidence for myocardial TNC expression in patients with DCM. Second, endomyocardial biopsy specimens may have some sampling errors. We used a transcatheter method to collect biopsy samples from three to five different sites in the RV septum. Evaluation of TNC accumulation was performed on all pieces collected, thus mitigating sampling error. However, there are advantages to evaluating myocardial deposition of the extracellular matrix by direct collection of myocardium by endomyocardial biopsy. Third, immunohistochemical analysis based on the use of an image processing program would be able to provide semiquantitative results at best. Future quantitative evaluation of

myocardial TNC protein or mRNA would strengthen our findings. Finally, the present study, through focusing on accumulation of myocardial TNC expression, could not completely clarify the mechanisms of LV remodeling and worse outcome in DCM. Other histological approaches, for example, evaluation of expression pattern or structure of TNC are worth considering in future.

In conclusion, we have shown that myocardial TNC expression was associated with lower occurrence of LV reverse remodeling and poor long-term outcome in patients with DCM.

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Conflict of Interest

None.

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Table 1. Patient characteristics by tenascin-C (TNC) area

	Total n = 123	High TNC n = 22	Low TNC n = 101	P value
Demographic characteristics				
Age, years	50.4 ± 14.5	45.9 ± 16.9	51.4 ± 13.9	0.186
Male	103 (84)	20 (91)	83 (82)	0.317
Body mass index, kg/m ²	23.8 ± 4.3	23.2 ± 4.8	23.9 ± 4.2	0.488
Current smoker	34 (28)	4 (18)	34 (34)	0.156
NYHA class III/VI	41 (33)	10 (46)	31 (31)	0.185
Duration of heart failure, months	21.6 ± 51.4	51.3 ± 92.2	15.1 ± 34.6	0.026
Systolic blood pressure, mmHg	116 ± 23	110 ± 22	117 ± 23	0.200
Diastolic blood pressure, mmHg	69 ± 12	67 ± 11	69 ± 12	0.706
Heart rate, b.p.m.	74 ± 15	75 ± 17	73 ± 14	0.639
Hypertension	49 (40)	9 (41)	40 (40)	0.910
Diabetes mellitus	19 (15)	8 (36)	11 (11)	0.003
Dyslipidemia	63 (51)	11 (50)	52 (52)	0.900
Atrial fibrillation	28 (23)	8 (36)	20 (20)	0.095
Left bundle branch block	16 (13)	4 (18)	12 (12)	0.439
ICD/BiV-ICD	8 (7)	3 (14)	5 (5)	0.707
Laboratory findings				
Log BNP	2.26 ± 0.59	2.59 ± 0.38	2.19 ± 0.61	0.003
HbA _{1c} , %	6.1 ± 0.9	6.2 ± 1.0	6.0 ± 0.9	0.455
Hemoglobin, g/dl	14.3 ± 1.9	14.2 ± 1.2	14.3 ± 2.0	0.905
eGFR, mL/min/1.73m ²	56.2 ± 20.9	55.2 ± 19.9	56.4 ± 21.2	0.448
Serum sodium, mEq/L	138 ± 4	139 ± 3	139 ± 5	0.577
Medication on admission				
β-blocker	53 (43)	12 (55)	41 (41)	0.233
ACE inhibitor/ARB	77 (63)	16 (73)	61 (60)	0.281
Loop diuretic	81 (66)	18 (82)	63 (62)	0.083
Aldosterone inhibitor	50 (41)	13 (59)	37 (37)	0.057
Sulphonylurea	1 (1)	0 (0)	1 (1)	0.641
Glinide	2 (2)	0 (0)	2 (2)	0.507
Insulin	2 (2)	1 (5)	1 (1)	0.234
Medication at discharge				
β-blocker	109 (89)	20 (91)	89 (88)	0.710
ACE inhibitor/ARB	98 (80)	21 (95)	77 (76)	0.043
Loop diuretic	78 (63)	19 (86)	59 (58)	0.014
Aldosterone inhibitor	62 (50)	15 (68)	47 (47)	0.067
Sulphonylurea	1 (1)	0 (0)	1 (1)	0.641

Glinide	2 (2)	1 (5)	1 (1)	0.234
Insulin	3 (2)	1 (5)	2 (2)	0.481
Echocardiographic findings				
LVEDD, mm	68.5 ± 8.4	70.2 ± 8.0	68.1 ± 8.5	0.179
LVESD, mm	59.4 ± 8.9	60.9 ± 9.3	59.1 ± 8.8	0.411
LVEF, %	25.2 ± 8.0	25.7 ± 8.0	25.1 ± 8.1	0.932
LAD, mm	46.1 ± 7.1	47.6 ± 6.7	45.8 ± 7.2	0.213
RVD, mm	29.9 ± 6.1	32.0 ± 6.9	29.4 ± 5.9	0.102
Mitral regurgitation	1.3 ± 0.9	1.3 ± 0.8	1.2 ± 0.9	0.579
Restrictive filling pattern	23 (19)	8 (36)	15 (15)	0.020
Hemodynamic data				
Mean PCWP, mmHg	12.1 ± 8.1	16.8 ± 8.6	11.1 ± 7.6	0.003
Mean PA, mmHg	20.0 ± 10.1	25.9 ± 10.1	18.7 ± 9.6	<0.001
Cardiac index, L/min/m ²	2.4 ± 0.6	2.2 ± 0.6	2.4 ± 0.6	0.171
Heart failure risk score	19.8 ± 6.6	22.0 ± 7.0	19.4 ± 6.4	0.116
Collagen area	14.4 ± 7.7	20.5 ± 10.5	13.1 ± 6.2	0.001

TNC = tenascin-C; NYHA = New York Heart Association; ICD = implantable cardioverter defibrillator; BiV-ICD = biventricular implantable cardioverter defibrillator; BNP = brain natriuretic peptide; Hb_{A1C} = hemoglobin A1c; eGFR = estimated glomerular filtration rate; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; LAD = left atrial diameter; RVD = right ventricular diameter; PCWP = pulmonary capillary wedge pressure; PA = pulmonary artery pressure.

Values are mean ± SD or n (%)

Table 2. Univariable and multivariable Cox regression analysis for death

	Univariable		Multivariable	
	HR (95%CI)	p value	HR (95% CI)	p value
Age	0.990 (0.948 – 1.033)	0.635	—	—
Body mass index	1.067 (0.937 – 1.215)	0.329	—	—
Current smoker	0.401 (0.085 – 1.889)	0.248	—	—
NYHA class III/IV	0.923 (0.238 – 3.577)	0.908	—	—
Duration of heart failure	1.008 (1.000 – 1.016)	0.063	—	—
Heart rate	0.987 (0.944 – 1.032)	0.566	—	—
Systolic blood pressure	0.955 (0.918 – 0.993)	0.020	0.981 (0.941 – 1.023)	0.367
Diabetes mellitus	5.298 (1.534 – 18.302)	0.008	4.323 (0.652 – 28.673)	0.129
ICD/BiV-ICD	15.115 (2.912 – 78.457)	0.001	7.801 (0.287 – 212.335)	0.223
Log BNP	6.728 (1.725 – 26.242)	0.006	6.661 (0.668 – 66.395)	0.106
Hemoglobin	0.904 (0.649 – 1.259)	0.904	—	—
eGFR	0.999 (0.967 – 1.032)	0.941	—	—
β-blocker at discharge	0.446 (0.095 – 2.104)	0.308	—	—
ACE inhibitor/ARB at discharge	0.558 (0.144 – 2.161)	0.398	—	—
Aldosterone inhibitor at discharge	1.363 (0.384 – 4.838)	0.631	—	—
Loop diuretics at discharge	1.783 (0.370 – 8.585)	0.471	—	—
LVEDDI	0.986 (0.880 – 1.104)	0.802	—	—
LVEF	1.028 (0.953 – 1.109)	0.473	—	—
Mitral regurgitation	1.770 (0.841 – 3.723)	0.132	—	—
Restrictive filling pattern	1.121 (0.233 – 5.400)	0.886	—	—
Heart failure risk score	0.986 (0.908 – 1.103)	0.986	—	—
Collagen area	1.089 (1.007 – 1.178)	0.032	1.016 (0.894 – 1.154)	0.808
Tenascin-C area	1.502 (1.243 – 1.814)	<0.001	1.347 (1.026 – 1.768)	0.032

HR = hazard ratio; CI = confidence interval; BiV-ICD = biventricular implantable

cardioverter defibrillator; BNP = brain natriuretic peptide; eGFR = estimated glomerular

filtration rate; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; LVEDDI = Left ventricular end-diastolic diameter index; LVEF = Left ventricular ejection fraction.

Table 3. Echocardiographic parameters at baseline and follow-up

	High TNC (n = 15)			Low TNC (n = 79)		
	Baseline	Follow-up	P value	Baseline	Follow-up	P value
LVEDD, mm	69.5 ± 8.6	64.7 ± 8.3	0.161	68.6 ± 9.2	53.4 ± 10.6	<0.001
LVESD, mm	59.9 ± 9.6	54.4 ± 8.4	0.174	59.7 ± 9.5	46.6 ± 12.6	<0.001
IVST, mm	8.8 ± 2.8	8.7 ± 2.7	0.928	8.7 ± 2.2	9.2 ± 2.1	0.094
PWT, mm	9.1 ± 2.2	8.8 ± 1.3	0.928	9.1 ± 2.1	9.2 ± 2.0	0.695
LVEF, %	26.6 ± 8.2	29.8 ± 8.2	0.285	24.8 ± 8.2	39.8 ± 13.4	<0.001
RVD, mm	32.5 ± 7.3	31.2 ± 6.6	0.780	29.5 ± 5.8	28.6 ± 5.0	0.290
E, cm/s	82.7 ± 29.3	68.2 ± 22.2	0.183	70.1 ± 28.6	63.8 ± 22.9	0.095
E/A	2.1 ± 1.0	1.1 ± 0.7	0.229	1.1 ± 0.6	1.0 ± 0.6	0.840
DcT, msec	145 ± 70	177 ± 61	0.139	169 ± 56	207 ± 56	<0.001
LAD, mm	49.3 ± 6.6	44.8 ± 11.5	0.134	46.4 ± 7.1	41.7 ± 7.9	<0.001

TNC = tenascin-C; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; IVST = interventricular septum thickness; PWT = posterior left ventricular wall thickness; LVEF = left ventricular ejection fraction; RVD = right ventricular diameter; E = peak early diastolic transmitral filling velocity; E/A = peak early diastolic transmitral filling velocity/peak late transmitral filling velocity ratio; Dct = deceleration time; LAD = left atrial diameter.

Values are mean ± SD.

Table 4. Univariable and multivariable linear regression analysis of TNC area

	Univariable			Univariable	
	β coefficient	P value		β coefficient	P value
Age	-0.067	0.462	Aldosterone inhibitor on admission	0.185	0.041
Male	-0.010	0.911	Sulphonylurea on admission	-0.062	0.492
Body mass index	-0.016	0.862	Glinide on admission	-0.053	0.564
Current smoke	-0.150	0.097	Insulin on admission	0.031	0.737
NYHA class III/VI	0.129	0.156	β -blocker at discharge	0.104	0.253
Duration of heart failure	0.266	0.003	ACE inhibitor/ARB at discharge	0.090	0.320
Systolic blood pressure	-0.088	0.335	Loop diuretic at discharge	0.181	0.045
Diastolic blood pressure	-0.083	0.361	Aldosterone inhibitor at discharge	0.134	0.140
Heart rate	-0.017	0.850	Sulphonylurea at discharge	-0.062	0.492
Hypertension	0.013	0.888	Glinide at discharge	0.143	0.115
Diabetes mellitus	0.266	0.003	Insulin at discharge	-0.013	0.887
Dyslipidemia	0.008	0.931	LVEDD	0.017	0.852
Atrial fibrillation	0.258	0.004	LVESD	0.004	0.961
Left bundle branch block	0.117	0.198	LVEF	-0.195	0.089
ICD/BiV-ICD	0.192	0.033	RVD	0.100	0.278
Log BNP	0.267	0.003	Mitral regurgitation	0.018	0.841
Hb _{A1c}	0.055	0.556	Restrictive filling pattern	0.215	0.017
Hemoglobin	-0.018	0.847	Mean PCWP	0.254	0.005
eGFR	-0.155	0.087	Mean PA	0.288	0.001
Serum sodium	-0.142	0.118	Cardiac index	-0.206	0.026
β -blocker on admission	0.146	0.108	Heart failure risk score	0.216	0.017
ACE inhibitor/ARB on admission	0.069	0.447	Collagen area	0.329	<0.001
Loop diuretic on admission	0.138	0.129			

	Multivariable			Multivariable	
	β coefficient	P value		β coefficient	P value
Duration of heart failure	0.117	0.239	Restrictive filling pattern	0.099	0.270
Diabetes mellitus	0.199	0.034	Mean PCWP	-0.503	0.032
Atrial fibrillation	0.174	0.063	Mean PA	0.644	0.008
ICD/BiV-ICD	0.211	0.015	Cardiac index	0.013	0.890
Log BNP	-0.040	0.735	Heart failure risk score	0.162	0.093
Aldosterone inhibitor on admission	-0.072	0.444	Collagen area	0.128	0.209
Loop diuretic at discharge	0.180	0.050			

TNC = tenascin-C; NYHA = New York Heart Association; ICD = implantable cardioverter defibrillator; BiV-ICD = biventricular implantable cardioverter defibrillator; BNP = brain natriuretic peptide; Hb_{A1C} = hemoglobin A1c; eGFR = estimated glomerular filtration rate; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; LAD = left atrial diameter; RVD = right ventricular diameter; PCWP = pulmonary capillary wedge pressure; PA = pulmonary artery pressure.

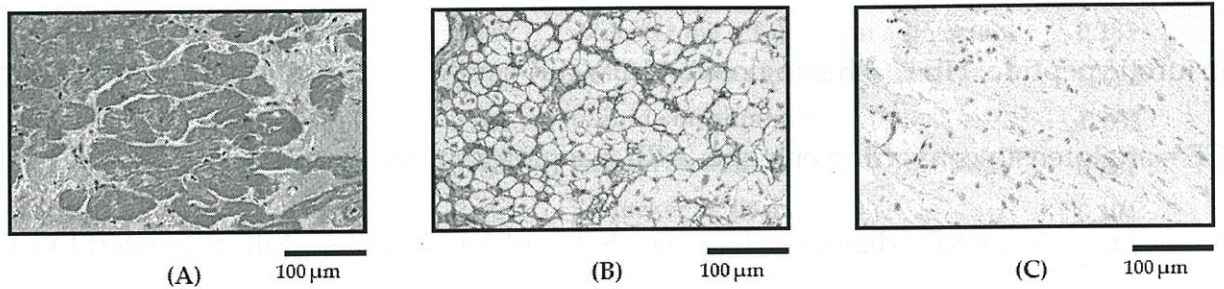


Figure 1. Representative microscopic images of biopsy specimens

(A) Collagen area (blue-stained area) stained with Masson's trichrome staining was measured as 30.5%. (B) Tenascin-C (TNC)-positive area in immunohistochemical staining for TNC was measured as 9.9%, falling in the high TNC group. (C) TNC-positive area in immunohistochemical staining for TNC was measured as 0.5%, falling in the low TNC group.

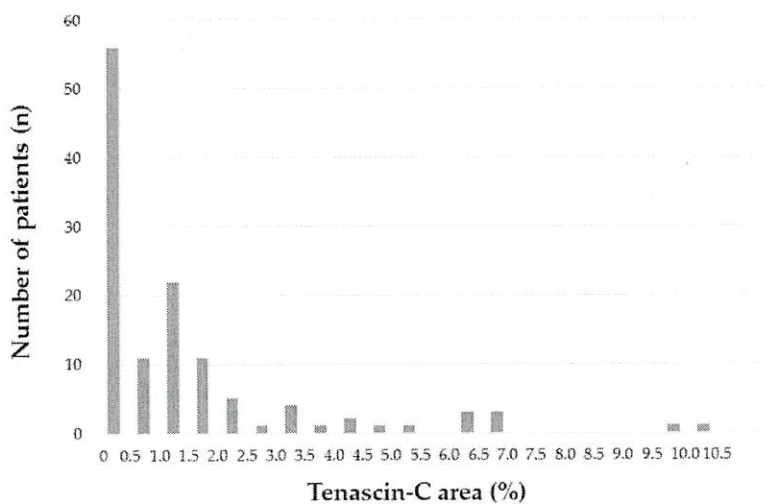


Figure 2. Distribution of tenascin-C area

Mean tenascin-C area was $1.4 \pm 2.0\%$, ranging from 0.1 to 10.4% with more than 80% of all patients showing tenascin-C area $< 2.0\%$.

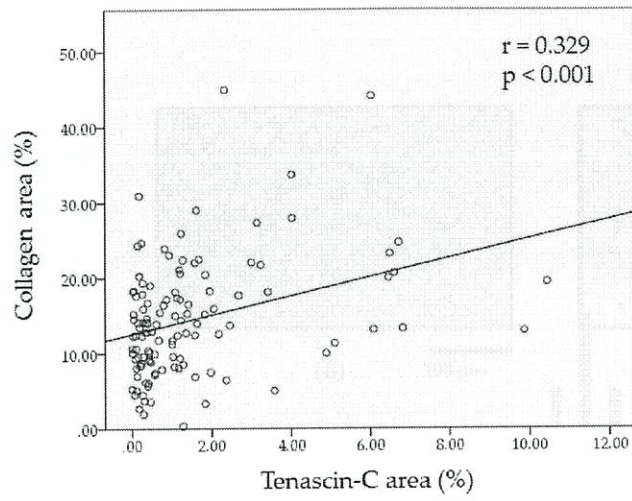


Figure 3. Correlation between tenascin-C area and collagen area

Tenascin-C area was positively correlated with collagen area ($r = 0.329$, $P < 0.001$).

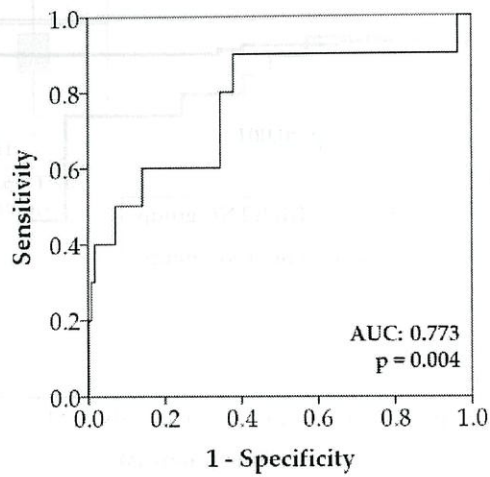


Figure 4. Receiver operating characteristic analysis of tenascin-C area to predict death

The optimal cut-off value of tenascin-C area to predict death in patients with dilated cardiomyopathy was determined to be 2.3% based on the receiver operating characteristic curve (sensitivity = 0.600, specificity = 0.858, area under the curve [AUC] = 0.773, P = 0.004) in these study subjects.

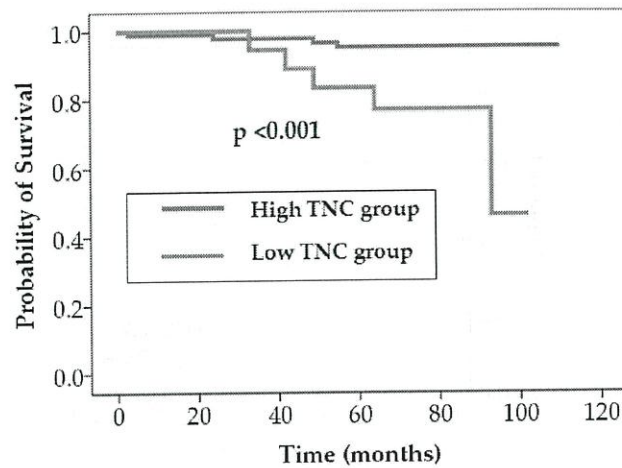


Figure 5. Kaplan-Meier curves for all-cause mortality

Event-free survival was calculated for the high tenascin-C (TNC) group (n = 22) and low TNC group (n = 101) by Kaplan-Meier method. Survival rate was significantly decreased in the high TNC group compared with the low TNC group.

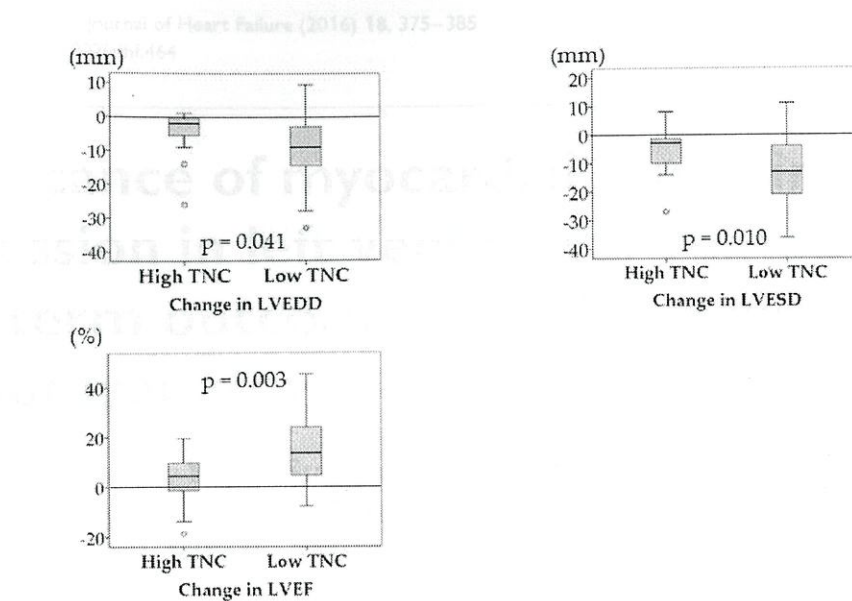


Figure 6. Changes in left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), and left ventricular ejection fraction (LVEF) in high tenascin-C (TNC) group and low TNC group

The bottom and top of the box are the first and third quartiles, and the band inside the box is the median. The ends of the whiskers denote 1.5 interquartile range of the lower and upper quartiles. The circles represent outliers. Changes in LVEDD (-9.2 ± 8.6 vs. -4.9 ± 7.2 mm) and LVESD (-13.0 ± 10.4 vs. -5.5 ± 8.5 mm) were significantly smaller in the low tenascin-C (TNC) group compared with the high TNC group. The change in LVEF was significantly smaller in the high TNC group (3.2 ± 10.8 vs. $15.0 \pm 12.6\%$). These data suggest that the high TNC group was associated with lower occurrence of reverse remodeling in patients with dilated cardiomyopathy.

Significance of myocardial tenascin-C expression in left ventricular remodelling and long-term outcome in patients with dilated cardiomyopathy

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Aim	Dilated cardiomyopathy (DCM) has a variety of causes, and no useful approach to predict left ventricular (LV) remodelling and long-term outcome has yet been established. Myocardial tenascin-C (TNC) is known to appear under pathological conditions, possibly to regulate cardiac remodelling. The aim of this study was to clarify the significance of myocardial TNC expression in LV remodelling and the long-term outcome in DCM.
Methods and results	One hundred and twenty-three consecutive DCM patients who underwent endomyocardial biopsy for initial diagnosis were studied. Expression of TNC in biopsy sections was analysed immunohistochemically to quantify the ratio of the TNC-positive area to the whole myocardial tissue area (TNC area). Clinical parameters associated with TNC area were investigated. The patients were divided into two groups based on receiver operating characteristic analysis of TNC area to predict death: high TNC group with TNC area $\geq 2.3\%$ (22 patients) and low TNC group with TNC area $< 2.3\%$ (101 patients). High TNC was associated with diabetes mellitus. Comparing echocardiographic findings between before and 9 months after endomyocardial biopsy, the low TNC group was associated with decreased LV end-diastolic diameter and increased LV ejection fraction, whereas the high TNC group was not. Survival analysis revealed a worse outcome in the high TNC group than in the low TNC group ($P < 0.001$). Multivariable Cox regression analysis revealed that TNC area was independently associated with poor outcome (HR = 1.347, $P = 0.032$).
Conclusions	Increased myocardial TNC expression was associated with worse LV remodeling and long-term outcome in DCM.
Keywords	Dilated cardiomyopathy • Heart failure • Tenascin-C • Left ventricular remodelling • Prognosis

Introduction

Dilated cardiomyopathy (DCM) is characterized by ventricular enlargement and impaired contractile function. The prognosis of DCM remains unfavourable, with a high incidence of sudden cardiac death and heart failure, and an estimated overall 1-year

mortality rate between 10% and 30%, and some patients with DCM require heart transplantation.^{1,2} Because of the widely diverse background characteristics of patients with DCM, the outcome of these patients is almost unpredictable, even with a variety of examinations, including blood tests, electrocardiography, chest X-ray, echocardiography, scintigraphy, and cardiac catheterization.

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Therefore, new prognostic indicators to stratify the future risk of DCM are strongly awaited.

Tenascin-C (TNC), a large hexameric glycoprotein, is known to appear in the extracellular matrix specifically following tissue injury or tumour formation where the particular organ is undergoing remodelling.³ As regards the cardiac system, TNC is synthesized and expressed under pathological conditions in myocardial infarction, acute myocarditis, and DCM, suggesting a potential impact on the progression of cardiovascular diseases.^{4,5} For example, TNC exacerbated heart failure by acceleration of left ventricular (LV) remodelling and an increase in fibrosis after myocardial infarction in a mouse model.⁶ However, the effect of myocardial TNC expression with LV remodelling and long-term outcome in DCM has not yet been fully investigated. Similarly, the clinical background in which expression of TNC is increased in the myocardium has not been elucidated, although TNC is known to be synthesized by interstitial fibroblasts under inflammatory stimulation.³

The objective of this study was to investigate the association of myocardial TNC expression with LV remodelling and the long-term outcome. In addition, we examined the clinical factors of patients predisposed to increased tissue TNC expression in DCM.

Methods

Study population

Patients who were diagnosed with DCM in our hospital between 2005 and 2008 with impaired contractile function with left ventricular ejection fraction (LVEF) <40% and left ventricular end-diastolic diameter (LVEDD) >55 mm were screened for the study. All patients underwent clinical evaluation, physical examination, 12-lead electrocardiography, laboratory tests, echocardiography, and cardiac catheterization, including coronary angiography and right ventricular (RV) septal endomyocardial biopsy for initial diagnosis. All patients underwent RV endomyocardial biopsy according to the indications in the American Heart Association, American College of Cardiology, and European Society of Cardiology scientific statement, mainly to exclude myocarditis or secondary cardiomyopathy.⁷ Endomyocardial biopsy was performed after obtaining written informed consent with information about the procedure and the risk of endomyocardial biopsy. Because medical therapy was optimized during hospitalization in most of the patients, information about the use of medication for heart failure such as β -blockers, angiotensin converting enzyme (ACE)-inhibitors or angiotensin receptor blockers (ARBs), and loop diuretics was collected separately on admission and at discharge. Patients with hypertrophic cardiomyopathy, ischaemic cardiomyopathy, valvular heart disease, relevant (LV) hypertrophy suggested to be caused by hypertensive heart disease, or secondary cardiomyopathy such as that caused by sarcoidosis or amyloidosis were excluded. Patients with a history of uncontrolled hypertension or LV assist device implantation were also excluded.

Study protocol

Information about the patients' baseline characteristics, such as age, gender, body mass index, presenting symptoms, blood pressure, heart

rate, previous comorbid conditions, and laboratory data (e.g. BNP, haemoglobin, serum creatinine), were collected from their medical records. Data on medication were collected both on admission and at discharge. Sections from stored paraffin-embedded RV septal tissue were stained with a specific antibody to TNC to evaluate TNC expression in the myocardium. Echocardiography was performed before and at 9 months after RV biopsy. Survival data were also collected in order to perform survival analysis. This study was performed according to the Helsinki Declaration and was approved by the ethics committee of our institution.

Heart failure risk score

To evaluate heart failure severity, we used heart failure risk score accessible by the website <http://www.heartfailure-risk.org>.⁸ This is the generalizable easily used integer risk score for mortality in patients with heart failure, including 13 independent predictors: age, LVEF, New York Heart Association (NYHA) class, serum creatinine, diabetes mellitus, β -blocker, systolic blood pressure, body mass index, time since diagnosis, current smoker, chronic obstructive pulmonary disease, male gender, and ACE inhibitor or ARB.

Echocardiography

Echocardiographic parameters were measured according to the recommendations of the American Society of Echocardiography.⁹ The LVEDD, left ventricular end-systolic diameter (LVESD), thickness of the interventricular septum and posterior ventricular wall, and left atrial diameter were obtained from M-mode or two-dimensional images of parasternal long axis views. Left ventricular end-diastolic diameter index (LVEDDI) was calculated as LVEDD divided by body surface area. LVEF was calculated by the Teichholz formula.¹⁰ Mitral regurgitation was semiquantitatively graded from 1 to 4 considering the regurgitant area at colour Doppler imaging. Peak early (E), late (A) diastolic transmitral filling velocities, and deceleration time of E were measured, and E/A ratio was calculated. Restrictive filling pattern was defined as the E-wave deceleration time of <115 ms.¹¹

Cardiac catheterization

Patients underwent diagnostic cardiac catheterization. Coronary angiography was performed to exclude ischaemic heart disease, and right heart catheterization was performed to obtain haemodynamic data including cardiac output, pulmonary capillary wedge pressure (PCWP), and pulmonary artery pressure (PA). Endomyocardial biopsy was also performed, and three to five pieces of ventricular tissue were obtained from different locations in the septal wall of the right ventricle using disposable biopsy forceps (Technowood Co., Ltd., Tokyo, Japan). The specimens were immediately fixed with 10% buffered formalin, embedded in paraffin, serially sectioned and stained with haematoxylin and eosin staining. Masson's trichrome staining was also performed to detect fibrosis.

Immunohistochemistry for tenascin-C

Immunohistochemical staining on formalin-fixed paraffin-embedded sections was performed using an autostainer Leica Bond-III (Leica Biosystems, Melbourne, Australia) according to the manufacturer's protocol. For antigen retrieval, slides were treated with Enzyme 1 using a Bond Enzyme Pretreatment Kit (AR9551;

Table 1 Patient characteristics by tenascin-C (TNC) area

	Total, n = 123	High TNC, n = 22	Low TNC, n = 101	P-value
Demographic characteristics				
Age, years	50.4 ± 14.5	45.9 ± 16.9	51.4 ± 13.9	0.186
Male	103 (84)	20 (91)	83 (82)	0.317
Body mass index, kg/m ²	23.8 ± 4.3	23.2 ± 4.8	23.9 ± 4.2	0.488
Current smoker	34 (28)	4 (18)	34 (34)	0.156
NYHA class III/VI	41 (33)	10 (46)	31 (31)	0.185
Duration of heart failure, months	21.6 ± 51.4	51.3 ± 92.2	15.1 ± 34.6	0.026
Systolic blood pressure, mmHg	116 ± 23	110 ± 22	117 ± 23	0.200
Diastolic blood pressure, mmHg	69 ± 12	67 ± 11	69 ± 12	0.706
Heart rate, b.p.m.	74 ± 15	75 ± 17	73 ± 14	0.639
Hypertension	49 (40)	9 (41)	40 (40)	0.910
Diabetes mellitus	19 (15)	8 (36)	11 (11)	0.003
Dyslipidaemia	63 (51)	11 (50)	52 (52)	0.900
Atrial fibrillation	28 (23)	8 (36)	20 (20)	0.095
Left bundle branch block	16 (13)	4 (18)	12 (12)	0.439
ICD/BiV-ICD	8 (7)	3 (14)	5 (5)	0.707
Laboratory findings				
Log BNP	2.26 ± 0.59	2.59 ± 0.38	2.19 ± 0.61	0.003
HbA _{1c} , %	6.1 ± 0.9	6.2 ± 1.0	6.0 ± 0.9	0.455
Haemoglobin, g/dl	14.3 ± 1.9	14.2 ± 1.2	14.3 ± 2.0	0.905
eGFR, mL/min. 1.73 m ²	56.2 ± 20.9	55.2 ± 19.9	56.4 ± 21.2	0.448
Serum sodium, mEq/L	138 ± 4	139 ± 3	139 ± 5	0.577
Medication on admission				
β-blocker	53 (43)	12 (55)	41 (41)	0.233
ACE inhibitor/ARB	77 (63)	16 (73)	61 (60)	0.281
Loop diuretic	81 (66)	18 (82)	63 (62)	0.083
Aldosterone inhibitor	50 (41)	13 (59)	37 (37)	0.057
Sulphonylurea	1 (1)	0 (0)	1 (1)	0.641
Glinide	2 (2)	0 (0)	2 (2)	0.507
Insulin	2 (2)	1 (5)	1 (1)	0.234
Medication at discharge				
β-blocker	109 (89)	20 (91)	89 (88)	0.710
ACE inhibitor/ARB	98 (80)	21 (95)	77 (76)	0.043
Loop diuretic	78 (63)	19 (86)	59 (58)	0.014
Aldosterone inhibitor	62 (50)	15 (68)	47 (47)	0.067
Sulphonylurea	1 (1)	0 (0)	1 (1)	0.641
Glinide	2 (2)	1 (5)	1 (1)	0.234
Insulin	3 (2)	1 (5)	2 (2)	0.481
Echocardiographic findings				
LVEDD, mm	68.5 ± 8.4	70.2 ± 8.0	68.1 ± 8.5	0.179
LVESD, mm	59.4 ± 8.9	60.9 ± 9.3	59.1 ± 8.8	0.411
LVEF, %	25.2 ± 8.0	25.7 ± 8.0	25.1 ± 8.1	0.932
LAD, mm	46.1 ± 7.1	47.6 ± 6.7	45.8 ± 7.2	0.213
RVD, mm	29.9 ± 6.1	32.0 ± 6.9	29.4 ± 5.9	0.102
Mitral regurgitation	1.3 ± 0.9	1.3 ± 0.8	1.2 ± 0.9	0.579
Restrictive filling pattern	23 (19)	8 (36)	15 (15)	0.020
Haemodynamic data				
Mean PCWP, mmHg	12.1 ± 8.1	16.8 ± 8.6	11.1 ± 7.6	0.003
Mean PA, mmHg	20.0 ± 10.1	25.9 ± 10.1	18.7 ± 9.6	<0.001
Cardiac index, L/min.m ²	2.4 ± 0.6	2.2 ± 0.6	2.4 ± 0.6	0.171
Heart failure risk score	19.8 ± 6.6	22.0 ± 7.0	19.4 ± 6.4	0.116
Collagen area, %	14.4 ± 7.7	20.5 ± 10.5	13.1 ± 6.2	0.001

TNC, tenascin-C; NYHA, New York Heart Association; ICD, implantable cardioverter defibrillator; BiV-ICD, biventricular implantable cardioverter defibrillator; BNP, brain natriuretic peptide; HbA_{1c}, haemoglobin A_{1c}; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; RVD, right ventricular diameter; PCWP, pulmonary capillary wedge pressure; PA, pulmonary artery pressure.

Values are mean ± SD or n (%).

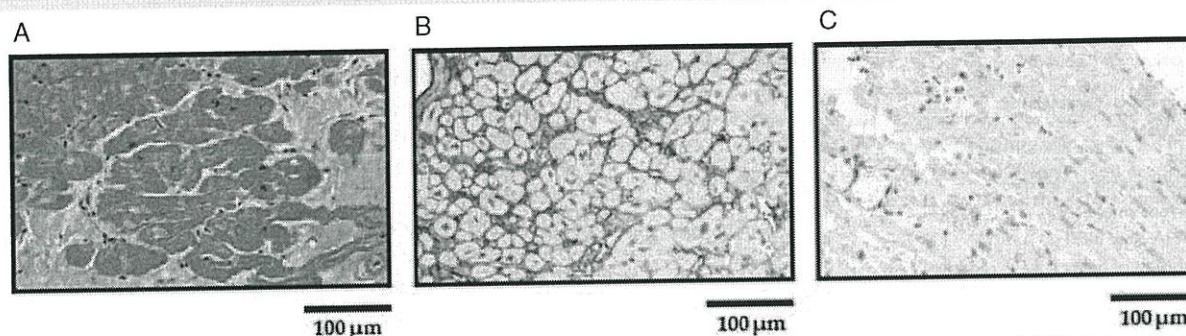


Figure 1 Representative microscopic images of biopsy specimens. (A) Collagen area (blue-stained area) stained with Masson's trichrome was measured as 30.5%. (B) Tenascin-C (TNC)-positive area in immunohistochemical staining for TNC was measured as 9.9%, falling in the high TNC group. (C) TNC-positive area in immunohistochemical staining for TNC was measured as 0.5%, falling in the low TNC group.

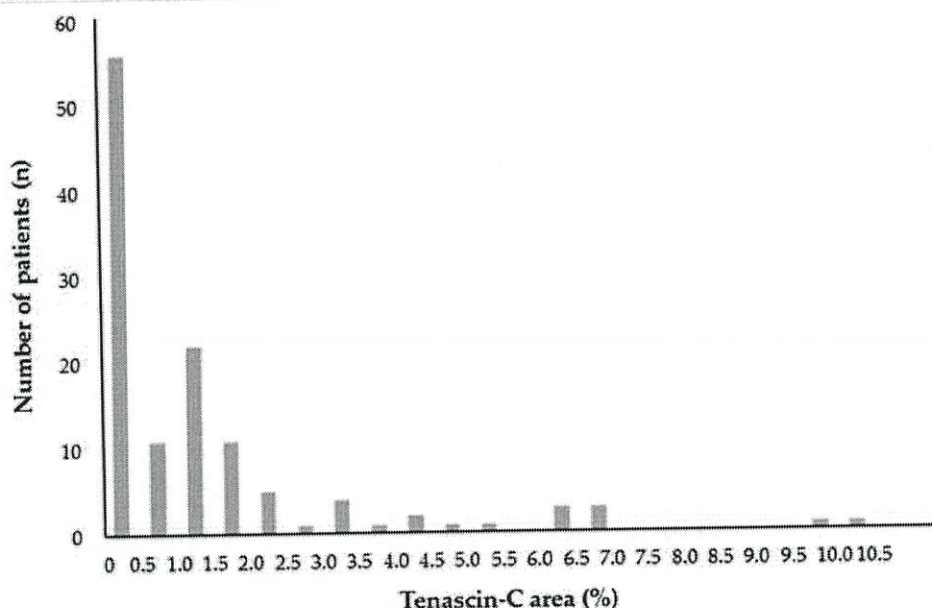


Figure 2 Distribution of tenascin-C area. Mean tenascin-C area was $1.4 \pm 2.0\%$, ranging from 0.1 to 10.4% with more than 80% of all patients showing a tenascin-C area $<2.0\%$.

Leica Biosystems, Newcastle-upon-Tyne, UK) for 5 min at 37°C . Slides were then incubated for 15 min at room temperature with a commercially available mouse monoclonal antibody for TNC (4F10TT, 1:1000 dilution; Immuno-Biological Laboratory Co., Ltd., Gunma, Japan), which recognizes epidermal growth factor (EGF)-like domain, constitutive sites of the TNC molecules.¹² Antibody detection and counterstaining with haematoxylin were performed using Bond Polymer Refine Detection Kit (DS9800; Leica Biosystems). Non-immunized mouse IgG (X0931, 1:1000 dilution; Dako, Glostrup, Denmark) was substituted as a negative control for the primary antibody against TNC to exclude possible false-positive responses from the secondary antibody or from non-specific binding of IgG.

Analyses of myocardial collagen accumulation and tenascin-C expression

Myocardial collagen accumulation and TNC expression were semi-quantitatively measured in a blinded manner. Photomicrographs were taken in five different randomly-selected high-power fields in the low-magnification whole view of three to five pieces obtained by endomyocardial biopsy. Images were analysed with National Institutes of Health imaging software. The ratio of total blue-stained area without endocardium and blood vessels to whole myocardial area in Masson's trichrome-stained sections was calculated. The average value was taken to represent collagen accumulation and defined as the collagen area. Similarly, the TNC area was expressed as the average of the ratio of

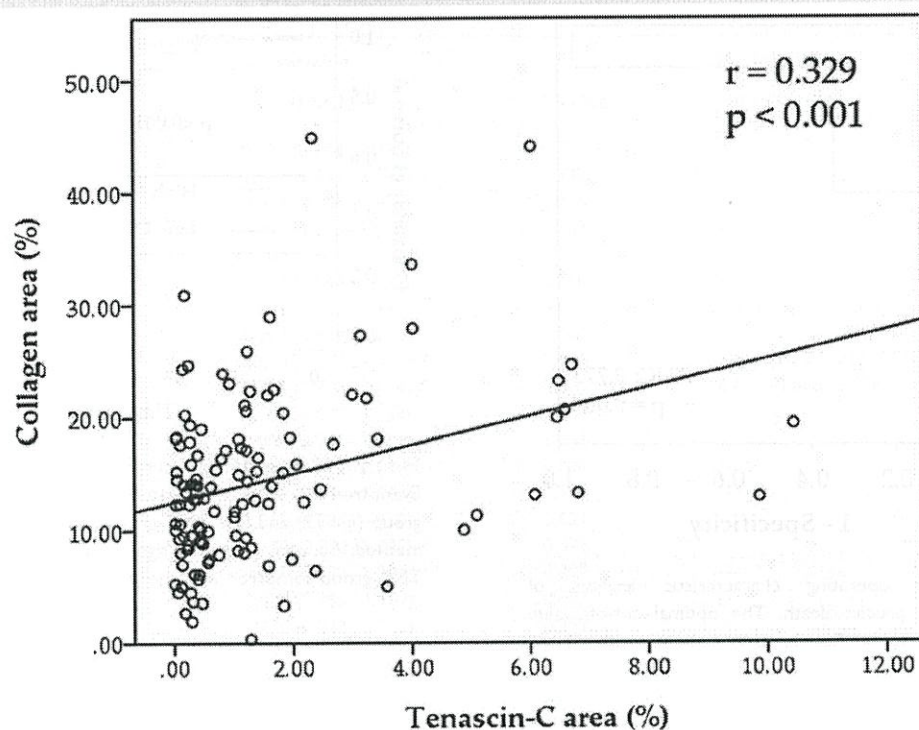


Figure 3 Correlation between tenascin-C area and collagen area. Tenascin-C area was positively correlated with collagen area ($r = 0.329$, $P < 0.001$).

TNC-positive area to the whole myocardial area in immunohistochemically stained sections.

Statistical analysis

Data were analysed using the Statistical Package for Social Sciences version 21.0 (SPSS Inc.; Chicago, IL, USA). All quantitative data were expressed as mean \pm SD. The statistical significance of differences was analysed using unpaired Student's *t*-test for parametric continuous variables, the Mann–Whitney *U*-test for non-parametric continuous variables, and paired *t*-test to compare echocardiographic parameters at baseline and follow-up. Categorical variables were compared using Fisher's exact test. Pearson's correlation analysis was performed to estimate correlations between TNC area and fibrosis area. The receiver operating characteristic curve was constructed to determine the cut-off values. For survival analysis, the outcome of interest was death, and other patients were censored at the time of LV assist device implantation or heart transplantation, or at the day of last follow-up. Event-free survival curves were constructed using the Kaplan–Meier method, and the statistical significance of differences between curves was assessed using the log-rank test. Cox proportional hazard analysis was performed with 22 clinical variables that are generally recognized parameters influencing heart failure prognosis [age, body mass index, current smoker, NYHA class III or IV, duration of heart failure, heart rate, systolic blood pressure, diabetes mellitus, implantable cardioverter defibrillator (ICD) or biventricular ICD, logBNP, haemoglobin, estimated glomerular filtration rate, β -blocker at discharge, ACE inhibitor or ARB at discharge, aldosterone inhibitor

at discharge, loop diuretic at discharge, LVEDDI, LVEF, mitral regurgitation, restrictive filling pattern, heart failure risk score, fibrosis area] and TNC area, and variables achieving $P < 0.05$ on univariable analysis were then tested in multivariable Cox analysis to determine which ones were significantly associated with death.⁸ Univariable linear regression analysis for TNC area was performed using all variables in the baseline characteristics, and then multivariable linear regression analysis was performed using the variables achieving $P < 0.05$ on univariable linear regression analysis to determine the factors associated with TNC area. Values of $P < 0.05$ were considered statistically significant.

Results

Patient clinical characteristics

A total of 138 consecutive patients were finally diagnosed with DCM, and of these, 123 patients with complete data collection were included in the study. On admission, 43% and 63% of patients were receiving β -blockers and ACE inhibitors/ARBs respectively, but, at discharge, most of the patients were receiving these key medications for heart failure, suggesting that medications were optimized during hospitalization (Table 1). During the follow-up period of 66 ± 35 months, nine patients (7.3%) died and eight patients (6.5%) underwent LV assist device implantation. No patient underwent heart transplantation before LV assist device implantation.

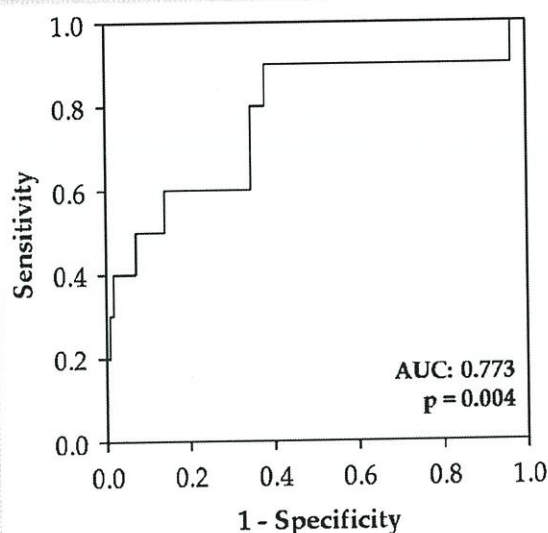


Figure 4 Receiver operating characteristic analysis of tenascin-C area to predict death. The optimal cut-off value of tenascin-C area to predict death in patients with dilated cardiomyopathy was determined to be 2.3% based on the receiver operating characteristic curve [sensitivity = 0.600, specificity = 0.858; area under the curve (AUC) = 0.773, $P = 0.004$] in these study subjects.

Myocardial collagen area and myocardial tenascin-C expression

A representative image of a Masson's trichrome-stained section is shown in Figure 1A (collagen area 30.5%). Collagen area ranged from 0.4 to 44.9% (mean $14.4 \pm 7.7\%$). Representative images of TNC-stained histological sections with high (Figure 1B, TNC area 9.9%) and low TNC (Figure 1C, TNC area 0.5%) expression are also shown. The distribution of TNC area is depicted in Figure 2. TNC area ranged from 0.1–10.4% (mean $1.4 \pm 2.0\%$), with more than 80% of all patients having TNC area $\leq 2\%$. The TNC area was positively correlated with collagen area ($r = 0.329$, $P < 0.001$; Figure 3). Based on receiver operating characteristic analysis, the optimal cut-off value of TNC area to predict all-cause death in patients with DCM was determined to be 2.3% (sensitivity = 0.600, specificity = 0.858, area under the curve = 0.773, $P = 0.004$; Figure 4) in these study subjects. We divided the patients into two groups using this cut-off value; (i) high TNC group with TNC area $\geq 2.3\%$ ($n = 22$, 18%), and (ii) low TNC group with TNC area $< 2.3\%$ ($n = 101$, 82%).

Patient characteristics and tenascin-C expression

Baseline characteristics were compared between the two groups (Table 1). Higher TNC expression was associated with longer duration of heart failure, higher incidence of diabetes mellitus and discharge, a loop diuretic at discharge and an ACE inhibitor

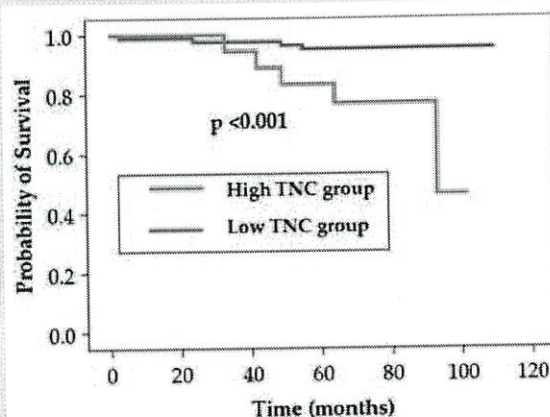


Figure 5 Kaplan–Meier curves for all-cause mortality. Event-free survival was calculated for the high tenascin-C (TNC) group ($n = 22$) and low TNC group ($n = 101$) by Kaplan–Meier method. Survival rate was significantly decreased in the high TNC group compared with the low TNC group.

or ARB at discharge. Mean PCWP, mean PA, plasma BNP level on admission, and collagen area in the high TNC group were higher than those in the low TNC group. However, baseline NYHA class, LV dimensions, LVEF, and heart failure risk score were not significantly different between the groups.

Association of tenascin-C expression with outcome

Kaplan–Meier survival curves demonstrated that the high TNC group had a poorer outcome than the low TNC group ($P < 0.001$; Figure 5). Univariable and multivariable Cox regression analyses were performed to determine predictive factors for death. Systolic blood pressure, diabetes mellitus, ICD or biventricular ICD, log BNP, collagen area, and TNC area with a P -value < 0.05 were selected by univariable analysis. Among these, multivariable analysis revealed that TNC area (hazard ratio = 1.347, $P = 0.032$) was an independent predictor of death (Table 2).

Left ventricular remodelling

One patient died and two others had an LV assist device implanted before follow-up echocardiography. Follow-up echocardiographic data were unavailable in 27 patients. A total of 94 patients (15 patients in the high TNC group and 79 patients in low TNC group) underwent follow-up echocardiography performed 9 ± 4 months after the diagnosis of DCM. We found that LVEDD, LVESD and LAD were significantly decreased ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively), and LVEF and deceleration time were significantly increased ($P < 0.001$ and $P < 0.001$, respectively) at follow-up compared with baseline in the low TNC group, while these values did not differ between baseline and follow-up in the high TNC group (Table 3). To compare LV remodelling between the

Table 2 Univariable and multivariable Cox regression analysis for death

	Univariable		Multivariable	
	HR (95%CI)	P-value	HR (95% CI)	P-value
Age	0.990 (0.948–1.033)	0.635	–	–
Body mass index	1.067 (0.937–1.215)	0.329	–	–
Current smoker	0.401 (0.085–1.889)	0.248	–	–
NYHA class III/IV	0.923 (0.238–3.577)	0.908	–	–
Duration of heart failure	1.008 (1.000–1.016)	0.063	–	–
Heart rate	0.987 (0.944–1.032)	0.566	–	–
Systolic blood pressure	0.955 (0.918–0.993)	0.020	0.981 (0.941–1.023)	0.367
Diabetes mellitus	5.298 (1.534–18.302)	0.008	4.323 (0.652–28.673)	0.129
ICD/BiV-ICD	15.115 (2.912–78.457)	0.001	7.801 (0.287–212.335)	0.223
Log BNP	6.728 (1.725–26.242)	0.006	6.661 (0.668–66.395)	0.106
Haemoglobin	0.904 (0.649–1.259)	0.904	–	–
eGFR	0.999 (0.967–1.032)	0.941	–	–
β-blocker at discharge	0.446 (0.095–2.104)	0.308	–	–
ACE inhibitor/ARB at discharge	0.558 (0.144–2.161)	0.398	–	–
Aldosterone inhibitor at discharge	1.363 (0.384–4.838)	0.631	–	–
Loop diuretics at discharge	1.783 (0.370–8.585)	0.471	–	–
LVEDDI	0.986 (0.880–1.104)	0.802	–	–
LVEF	1.028 (0.953–1.109)	0.473	–	–
Mitral regurgitation	1.770 (0.841–3.723)	0.132	–	–
Restrictive filling pattern	1.121 (0.233–5.400)	0.886	–	–
Heart failure risk score	0.986 (0.908–1.103)	0.986	–	–
Collagen area	1.089 (1.007–1.178)	0.032	1.016 (0.894–1.154)	0.808
Tenascin-C area	1.502 (1.243–1.814)	<0.001	1.347 (1.026–1.768)	0.032

HR, hazard ratio; CI, confidence interval; BiV-ICD, biventricular implantable cardioverter defibrillator; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEDDI, left ventricular end-diastolic diameter index; LVEF, left ventricular ejection fraction.

Table 3 Echocardiographic parameters at baseline and follow-up

	High TNC (n = 15)			Low TNC (n = 79)		
	Baseline	Follow-up	P-value	Baseline	Follow-up	P-value
LVEDD, mm	69.5 ± 8.6	64.7 ± 8.3	0.161	68.6 ± 9.2	53.4 ± 10.6	<0.001
LVESD, mm	59.9 ± 9.6	54.4 ± 8.4	0.174	59.7 ± 9.5	46.6 ± 12.6	<0.001
IVST, mm	8.8 ± 2.8	8.7 ± 2.7	0.928	8.7 ± 2.2	9.2 ± 2.1	0.094
PWT, mm	9.1 ± 2.2	8.8 ± 1.3	0.928	9.1 ± 2.1	9.2 ± 2.0	0.695
LVEF, %	26.6 ± 8.2	29.8 ± 8.2	0.285	24.8 ± 8.2	39.8 ± 13.4	<0.001
RVD, mm	32.5 ± 7.3	31.2 ± 6.6	0.780	29.5 ± 5.8	28.6 ± 5.0	0.290
E, cm/s	82.7 ± 29.3	68.2 ± 22.2	0.183	70.1 ± 28.6	63.8 ± 22.9	0.095
E/A	2.1 ± 1.0	1.1 ± 0.7	0.229	1.1 ± 0.6	1.0 ± 0.6	0.840
DcT, ms	145 ± 70	177 ± 61	0.139	169 ± 56	207 ± 56	<0.001
LAD, mm	49.3 ± 6.6	44.8 ± 11.5	0.134	46.4 ± 7.1	41.7 ± 7.9	<0.001

TNC, tenascin-C; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVST, interventricular septum thickness; PWT, posterior left ventricular wall thickness; LVEF, left ventricular ejection fraction; RVD, right ventricular diameter; E, peak early diastolic transmitral filling velocity; E/A, peak early diastolic transmitral filling velocity/peak late transmitral filling velocity ratio; DcT, deceleration time; LAD, left atrial diameter. Values are mean ± SD.

high and low TNC groups, we evaluated the changes in echocardiographic parameters from baseline to follow-up. Changes in LVEDD ($P = 0.041$) and LVESD ($P = 0.010$) were significantly smaller in the low TNC group, and change in LVEF ($P = 0.003$) was greater in the low TNC group compared with the high TNC group, suggesting that LV reverse remodelling was less prone to be induced during the follow-up period in the high TNC group (Figure 6).

Determinants of tenascin-C area

Univariable and multivariable linear regression analysis were performed to determine the background characteristics associated with the high TNC area. All variables in the baseline patient characteristics were selected for univariable analysis. Among these variables, diabetes mellitus, ICD or biventricular ICD, mean

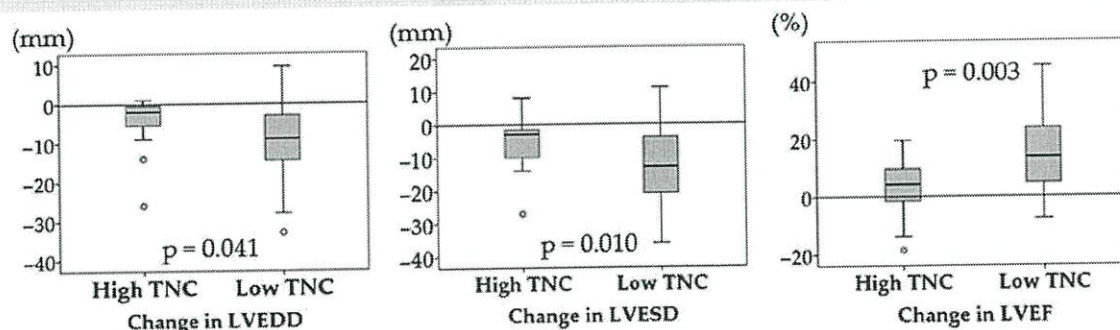


Figure 6 Changes in left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), and left ventricular ejection fraction (LVEF) in high tenascin-C (TNC) group and low TNC group. The bottom and top of the box are the first and third quartiles, and the band inside the box is the median. The ends of the whiskers denote 1.5 interquartile range of the lower and upper quartiles. The circles represent outliers. Changes in LVEDD (-9.2 ± 8.6 mm vs. -4.9 ± 7.2 mm) and LVESD (-13.0 ± 10.4 mm vs. -5.5 ± 8.5 mm) were significantly smaller in the low TNC group compared with the high TNC group. The change in LVEF was significantly smaller in the high TNC group ($3.2 \pm 10.8\%$ vs. $15.0 \pm 12.6\%$). These data suggest that the high TNC group was associated with lower occurrence of reverse remodelling in patients with dilated cardiomyopathy.

PCWP, and mean PA were shown to be independent determinants of high TNC area by multivariable analysis (Table 4).

Discussion

In the present study, we found that myocardial TNC expression, immunohistochemically stained and analysed semiquantitatively in RV biopsy samples, was a useful predictor of LV remodelling and long-term outcome.

The outcome of DCM varies greatly among individual cases and is closely associated with the degree of LV remodelling and whether cardiac function is improved by conventional therapy, including β -blockers and ACE-inhibitors.^{13,14} Left ventricular remodelling is affected by various kinds of neurohumoral and local factors such as the renin-angiotensin-aldosterone system, the adrenergic nervous system, increased oxidative stress, proinflammatory cytokines, and endothelin.^{13–17} However, the precise mechanisms that cause LV remodelling are still unclear.

Ventricular extracellular matrix proteins, which maintain cardiac structure and architecture in combination with myocytes, play a central role in LV remodelling in DCM.¹⁸ Recently, several studies have shown that TNC, one of the extracellular matrix component proteins, is expressed in association with several cardiovascular diseases.^{19,20} This large glycoprotein (>300 kDa) is expressed only during the development of the embryonic heart and not in the normal adult heart.²¹ However, when exposed to mechanical or ischaemic stress, it reappears in the heart. For example, TNC is expressed after myocardial infarction, in acute or chronic myocarditis, and in DCM in response to tissue injury and inflammation.^{4,5} Previously, serum TNC level was reported to be related to disease progression and to have prognostic significance in DCM. Serum measurement of TNC level, because of its less invasive nature, could serve as a potential clinical biomarker reflecting TNC expression.^{19,20,22} In the present study, by directly

staining myocardial biopsy specimens, we demonstrated that high TNC expression was related to a worse outcome and lower occurrence of reverse remodelling in patients with DCM. As TNC is not exclusively synthesized in the heart, but also in organs such as the liver and lungs,³ direct histological investigation of myocardial expression using a specific antibody appears to have great significance.

Tenascin-C has several functions that weaken cell adhesion, upregulate the expression and activity of matrix metalloproteinases, modulate inflammatory response, promote recruitment of myofibroblasts, and enhance fibrosis.³ In an experimental myocardial infarction model using genetically altered mice, TNC deficiency ameliorated collagen accumulation in the infarct border area, resulting in improved post-myocardial infarction cardiac function.⁶ Tenascin-C has the structural capability to bind and interact with other cells. This large extracellular matrix glycoprotein, consisting of EGF-like repeats, fibronectin III repeats, and a fibrinogen-like domain, has several biological effects. The antibody we used in the current study recognizes the EGF-like domain and is suitable to measure total myocardial TNC level, because the subsequent fibronectin III domain contains a splice variant region that varies considerably in its expression form. The EGF-like repeat domain interacts with the epidermal growth factor receptor; and the fibronectin III-like repeat domain binds integrins to promote adhesion and mediates cell activation via toll-like receptor 4, leading to sustained inflammation.²³ The unfavourable effects of TNC on ventricular remodelling in DCM might result from ongoing myocardial damage or inability to repair the failing heart because of sustained inflammation. There is increasing evidence that splicing variants of TNC, especially those containing the fibronectin III B or C domain, may influence tissue remodelling in heart failure. Serum level, as well as cardiac tissue deposition, of the B⁺ TNC variant was associated with mortality in DCM.²² In patients with heart failure, serum levels of B⁺ and C⁺ TNC were

Table 4 Univariable and multivariable linear regression analysis of tenascin-C (TNC) area

	Univariable		Multivariable	
	β -coefficient	P-value	β -coefficient	P-value
Age	-0.067	0.462	—	—
Male	-0.010	0.911	—	—
Body mass index	-0.016	0.862	—	—
Current smoker	-0.150	0.097	—	—
NYHA III/VI	0.129	0.156	—	—
Duration of heart failure	0.266	0.003	0.117	0.239
Systolic blood pressure	-0.088	0.335	—	—
Diastolic blood pressure	-0.083	0.361	—	—
Heart rate	-0.017	0.850	—	—
Hypertension	0.013	0.888	—	—
Diabetes mellitus	0.266	0.003	0.199	0.034
Dyslipidaemia	0.008	0.931	—	—
Atrial fibrillation	0.258	0.004	0.174	0.063
Left bundle branch block	0.117	0.198	—	—
ICD/BiV-ICD	0.192	0.033	0.211	0.015
Log BNP	0.267	0.003	-0.040	0.735
HbA _{1c}	0.055	0.556	—	—
Haemoglobin	-0.018	0.847	—	—
eGFR	-0.155	0.087	—	—
Serum sodium	-0.142	0.118	—	—
β -blocker on admission	0.146	0.108	—	—
ACE inhibitor/ARB on admission	0.069	0.447	—	—
Loop diuretic on admission	0.138	0.129	—	—
Aldosterone inhibitor on admission	0.185	0.041	-0.072	0.444
Sulphonylurea on admission	-0.062	0.492	—	—
Glinide on admission	-0.053	0.564	—	—
Insulin on admission	0.031	0.737	—	—
β -blocker at discharge	0.104	0.253	—	—
ACE inhibitor/ARB at discharge	0.090	0.320	—	—
Loop diuretic at discharge	0.181	0.045	0.180	0.050
Aldosterone inhibitor at discharge	0.134	0.140	—	—
Sulphonylurea at discharge	-0.062	0.492	—	—
Glinide at discharge	0.143	0.115	—	—
Insulin at discharge	-0.013	0.887	—	—
LVEDD	0.017	0.852	—	—
LVESD	0.004	0.961	—	—
LVEF	-0.195	0.089	—	—
RVD	0.100	0.278	—	—
Mitral regurgitation	0.018	0.841	—	—
Restrictive filling pattern	0.215	0.017	0.099	0.270
Mean PCWP	0.254	0.005	-0.503	0.032
Mean PA	0.288	0.001	0.644	0.008
Cardiac index	-0.206	0.026	0.013	0.890
Heart failure risk score	0.216	0.017	0.162	0.093
Collagen area	0.329	<0.001	0.128	0.209

TNC, tenascin-C; NYHA, New York Heart Association; ICD, implantable cardioverter defibrillator; BiV-ICD, biventricular implantable cardioverter defibrillator; BNP, brain natriuretic peptide; HbA_{1c}, haemoglobin A_{1c}; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; RVD, right ventricular diameter; PCWP, pulmonary capillary wedge pressure; PA, pulmonary artery pressure.

associated with heart chamber dilatation, physical performance, and BNP level, suggesting that they are possible biomarkers for disease severity.²⁴ Further immunohistochemical analysis for TNC splicing variants might provide additional valuable information related to LV remodelling.

Another intriguing finding of this study was the relevance of diabetes mellitus to myocardial TNC. Multivariable analysis showed that the presence of diabetes, as well as indices representing heart failure severity such as previous implantation of ICD or biventricular ICD, mean PCWP, and mean PA, was independently associated

with myocardial TNC expression. Diabetic cardiomyopathy is a disorder of heart muscle resulting from diabetes, the pathogenesis of which is yet to be clearly defined. Remodelling of cardiac extracellular matrix is known to be a key pathological feature of diabetic cardiomyopathy. Production of matricellular proteins is increased by hyperglycaemia- and hyperinsulinaemia-induced chronic inflammation, purportedly through transforming growth factor- β signalling.²⁵ In addition, persistent hyperglycaemia induces the formation of advanced glycation end-products, which is associated with increased production and reduced turnover of matricellular proteins.²⁶ Taking into consideration that TNC is likely to be upregulated in an inflammatory microenvironment, the increased myocardial expression of TNC in the present study might have been caused by stimulation of increased transforming growth factor- β associated with diabetes mellitus. We believe that this study also supports the pathophysiological significance of TNC in diabetic cardiomyopathy, in that TNC may contribute to the development of worsening heart function in diabetic patients. Furthermore, from a clinical aspect, it is suggested that preventing or adequately controlling diabetes mellitus might be able to reduce myocardial chronic inflammation, diminish myocardial TNC expression and consequently promote cardiac reverse remodelling in DCM patients.

In the present study, RV endomyocardial biopsy was selected to collect myocardial tissue samples, in order to minimize the procedural risk. It is controversial as to whether RV biopsy specimens are adequate for evaluation of LV remodelling in cardiomyopathy. We consider myocardial TNC measurement using RV myocardial samples is reasonable for the present study for two reasons. First, samples were obtained from the interventricular septum, which is considered common to the right ventricle and left ventricle. Second, progression of cardiac remodelling in DCM typically results in both RV and LV dysfunction at the same time. In fact, there is evidence that right ventricular dysfunction is correlated with LV dysfunction in patients with DCM.²⁷ We experienced difficulty in RV volumetric and functional assessment because of geometric complexity and asymmetry of the RV.²⁸ Cardiac magnetic resonance imaging might be an option to evaluate RV remodelling.

There are several limitations to this study. First, this was a retrospective study that included a relatively small number of patients. Follow-up echocardiography was not performed in all patients, although 76% of the study population did undergo this procedure. Larger prospective studies with complete follow-up are needed to establish evidence for myocardial TNC expression in patients with DCM. Second, endomyocardial biopsy specimens may have some sampling errors. We used a transcatheter method to collect biopsy samples from three to five different sites in the RV septum. Evaluation of TNC accumulation was performed on all pieces collected, thus mitigating sampling error. However, there are advantages to evaluating myocardial deposition of the extracellular matrix by direct collection of myocardium by endomyocardial biopsy. Third, immunohistochemical analysis based on the use of an image processing program would be able to provide semiquantitative results at best. Future quantitative evaluation of myocardial TNC protein or mRNA would strengthen our findings. Finally, the present study, through focusing on accumulation of myocardial TNC expression,

could not completely clarify the mechanisms of LV remodelling and worse outcome in DCM. Other histological approaches, for example, evaluation of expression pattern or structure of TNC are worth considering in future.

In conclusion, we have shown that myocardial TNC expression was associated with lower occurrence of LV reverse remodelling and poor long-term outcome in patients with DCM.

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